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Polypeptide backbone, C β and methyl group resonance assignments of the 24 kDa plectin repeat domain 6 from human protein plectin

Surya VSRK Pulavarti[#]

Department of Chemistry, The State University of New York at Buffalo, and Northeast Structural Genomics Consortium, Buffalo, NY 14260, USA Phone: (716) 645-4309, Fax: (716) 645-6963

Alexander Eletsky[#]

Department of Chemistry, The State University of New York at Buffalo, and Northeast Structural Genomics Consortium, Buffalo, NY 14260, USA Phone: (716) 645-4309, Fax: (716) 645-6963

Yuanpeng J. Huang

Center for Advanced Biotechnology and Medicine and Department of Molecular Biology and Biochemistry, Rutgers, The State University of New Jersey and Northeast Structural Genomics Consortium, Piscataway, NJ 08854, USA

Thomas B. Acton

Center for Advanced Biotechnology and Medicine and Department of Molecular Biology and Biochemistry, Rutgers, The State University of New Jersey and Northeast Structural Genomics Consortium, Piscataway, NJ 08854, USA

Rong Xiao

Center for Advanced Biotechnology and Medicine and Department of Molecular Biology and Biochemistry, Rutgers, The State University of New Jersey and Northeast Structural Genomics Consortium, Piscataway, NJ 08854, USA

John K. Everett

Center for Advanced Biotechnology and Medicine and Department of Molecular Biology and Biochemistry, Rutgers, The State University of New Jersey and Northeast Structural Genomics Consortium, Piscataway, NJ 08854, USA

Gaetano T. Montelione

Department of Biochemistry and Molecular Biology, Robert Wood Johnson Medical School, Rutgers, The State University of New Jersey, Piscataway NJ 08854, USA

Thomas Szyperski

Department of Chemistry, The State University of New York at Buffalo, and Northeast Structural Genomics Consortium, Buffalo, NY 14260, USA Phone: (716) 645-4309, Fax: (716) 645-6963

[#] These authors contributed equally to this work.

Abstract

The 500 kDa protein plectin is essential for the cytoskeletal organization of most mammalian cells and it is up-regulated in some types of cancer. Here, we report nearly complete sequence-specific polypeptide backbone, $^{13}\text{C}^\beta$ and methyl group resonance assignments for 24 kDa human plectin(4403-4606) containing the C-terminal plectin repeat domain 6.

Keywords

cytoskeletal linker protein; HCPIN; plakin repeat domain; plectin repeat domain; selective isotope labeling; structural genomics

Biological context

Plectin is a large 500-kDa ubiquitously expressed cytoskeletal scaffolding protein that primarily connects the intermediate filaments (IF) to cellular junctions and organelles, thereby playing an important role for both maintaining structural integrity of cells and signaling. Abnormal plectin expression results in disorders of skin and muscle tissue (Castanon et al. 2013). Moreover, Plectin was proposed as a biomarker for pancreatic ductal carcinoma and its aberrant expression may play a role in the manifestation of the disease (Shin et al. 2013). Plectin is also up-regulated other types of cancer such as colorectal adenocarcinoma, prostate cancer and head and neck squamous cell carcinoma.

Plectin domain organization (Figure 1a) consists of a central coiled-coil rod domain flanked by N- and C-terminal globular domains (Liu et al. 1996). The central rod is presumed to mediate the dimerization of plectin through coiled-coil interactions. The N-terminal segment includes an actin binding domain, consisting of two calponin homology domains, as well as a plakin domain which consists of spectrin repeats. The C-terminal segment is composed of six plectin repeat domains (PRD, also called “plakin repeat domains”) connected by linker sequences. Unlike most other types of polypeptide repeat domains that form extended structures with variable number of repeat units, PRDs are compact globular structures with a core of four 38- residue plectin repeats (PR) and a fifth truncated PR, flanked by polypeptide segments that may include additional PR-like sequence motifs (Choi et al. 2002).

24 kDa plectin repeat domain 6 (PRD6) of human plectin was selected for structure determination by the Northeast Structure Genomics Consortium (NESG; <http://www.nesg.org>; target ID: HR6354E) as a part of a biomedical theme, that is, the Human Cancer Interaction Protein Network (HCPIN) project (Huang et al. 2008). This project aims to provide extensive structure coverage for human cancer-associated proteins and their complexes in context of their interaction networks. Here we report nearly complete sequence-specific polypeptide backbone, C^β and methyl resonance assignments of PRD6. Those will enable us to solve the NMR structure based on ‘sparse constraints’ (Raman et al. 2010) complemented by methyl group NOEs.

Methods and Experiments

Sample preparation

Following established protocols (Jansson et al. 1996), Human plectin fragment 4303-4606 containing PRD6 was cloned into a pET15_NESG (Novagen) derivative yielding the plasmid HR6354E-4403-4606-15.3, which has been deposited in the PSI Materials Repository (<http://psimr.asu.edu/>) The resulting construct contained 11 additional non-native N-terminal residues (MGHHHHHSHM) to facilitate protein purification. Cells of the rare codon enhanced *Escherichia coli* strain BL21 (DE3) pMGK were transformed with the HR6354E-4403-4606-15.3 plasmid. Subsequently, three stable isotope labeled samples were prepared for the present NMR study: (i) [U - ^{13}C , ^{15}N]-PRD6 (PRD6-NC), (ii) [5% ^{13}C ; U - ^{15}N]-PRD6 (PRD6-NC5), (iii) [U - ^2H , ^{13}C , ^{15}N ; ^1H -Ile- δ^1 , Leu- δ , Val- γ]-PRD6 (PRD6-NCD-ILV). The PRD6-NC labeled sample was prepared using ($^{15}\text{NH}_4$) $_2\text{SO}_4$ and U - ^{13}C -glucose as sole nitrogen and carbon sources in the MJ9 medium. The uniformly ^{15}N - and 5% biosynthetically directed fractionally ^{13}C -labeled sample PRD6-NC5 was generated using a mixture of 95% natural abundance and 5% U - ^{13}C -glucose as a carbon source in the culture medium. The triple-labeled with the methyl groups of Val, Leu, Ile (δ^1) selectively protonated PRD6-NCD-ILV sample was prepared using ($^{15}\text{NH}_4$) $_2\text{SO}_4$ and U - ^{13}C -glucose with addition of [U - $^{13}\text{C}_4$, 3,3- $^2\text{H}_2$]- α -ketobutyrate [50 mg/L], [U - $^{13}\text{C}_5$, 3- $^2\text{H}_2$]- α -ketoisovalerate [CIL Inc] [100 mg/L] in deuterated MJ9 medium. All samples were purified using an AKTApurify system (GE Healthcare) with two-step protocol consisting of IMAC (HisTrap HP) chromatography, and gel filtration (HiLoad 26/60 Superdex 75) chromatography. The final yields of purified (i.e., > 98% homogenous by SDS-PAGE) PRD6-NC, PRD6-NC5 and PRD6-NCD-ILV were, respectively, about 20 mg/L, 14 mg/L and 15 mg/L. The integrity and isotope-enrichment of these samples was further validated by MALDI-TOF mass spectrometry. The final protein samples were prepared at concentrations of about 0.8-1 mM in 90% H_2O /10% D_2O solution containing 20 mM 2-(N-morpholino)ethanesulfonic acid (MES; pH 6.5), 100 mM NaCl, 10 mM Dithiothreitol (DTT), 5 mM CaCl_2 , 50 μM 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS), and 0.02% NaN_3 . An isotropic overall rotational correlation time of about ~13 ns was inferred from average backbone ^{15}N spin relaxation times, indicating that the protein is monomeric in solution. This was confirmed with analytical gel-filtration (Agilent Technologies) and static light scattering (Wyatt Technology Co).

NMR experiments

NMR experiments were acquired at 25 °C on Varian INOVA 600 and 750 MHz and Bruker Avance 900 MHz spectrometers equipped with cryogenic $^1\text{H}\{^{15}\text{N}, ^{13}\text{C}\}$ probes. The acquisition parameters for the experiments are provided in Table I. NMR spectra were processed using the program PROSA 6.4 and analyzed using the programs XEASY and CARRA. ^1H chemical shifts were referenced relative to internal DSS, and ^{13}C and ^{15}N chemical shifts were referenced indirectly via gyromagnetic ratios.

Assignments and data deposition

Sequence-specific polypeptide backbone (Figure 1b) and $^{13}\text{C}^\beta$ resonance assignments were obtained for the PRD6-NCD-ILV sample in a semi-automated fashion by analyzing TROSY based triple resonance experiments (Table I): 3D TROSY-HNCA, 3D TROSY-HN(CO)CA, 3D TROSY-HNCO, 3D TROSY-HN(CA)CO, 3D TROSY-HNCACB, and 3D TROSY-HN(COCA)CB (Cavanagh et al. 2006) using program Autoassign (Moseley et al. 2001) and extended by manual interactive spectral analysis. The assignments were confirmed by sequential NOEs observed in 3D [H]-NOESY-[NH/CH] and [(H)C]-NOESY-[CH] (Table I). Overall, sequence specific resonance assignments were obtained for > 97 % of the backbone and $^{13}\text{C}^\beta$ spins (99% H^N , 99% ^{15}N , 99% $^{13}\text{C}^\alpha$, 99% $^{13}\text{C}^\beta$ and 92 % of $^{13}\text{C}^\gamma$). Furthermore, 95% of the Ile δ^1 , Leu δ , and Val γ methyl group resonances (Fig. 1c) were assigned using the methyl out-and-back experiments (Tugarinov and Kay 2003) 3D HMC[CG1CBCA and 3D HMC[MCBCA in conjunction with 3D [H]-NOESY-[NH/CH] / [(H)C]-NOESY-[CH]. Chemical shifts measured for PRD6-NCD-ILV were corrected for deuterium isotope effects on the shifts, and all methyl resonance assignments were then confirmed by analysis of aliphatic (H)CCH and (H)CCH-TOCSY, and 3D [H]-NOESY-[NH/CH] acquired for PRD6-NC (Table I). Finally, stereo-specific assignments were obtained for 76% of the methyl groups of Val and Leu residues from 2D constant time (ct) [^{13}C , ^1H] HSQC spectra acquired with different ct delays for PRD6-NC5 (Table I).

The location of regular secondary structure elements (10 β -strands and 9 α -helices) predicted on the basis of the consensus chemical shift index CSI (Wishart and Sykes 1994) were confirmed by observation patterns of sequential and medium-range NOEs, and are in agreement with a known atomic resolution structure of desmoplakin C-terminal plaklin repeat domain (PDB 1LM5) exhibiting 62 sequence identity with PRD6 (Choi et al. 2002).

Chemical shifts were deposited in the BioMagResBank with BMRB accession number 19753.

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References

- Castanon MJ, Walko G, Winter L, Wiche G. Plectin-intermediate filament partnership in skin, skeletal muscle, and peripheral nerve. *Histochem Cell Biol.* 2013;140:33–53. [PubMed: 23748243]
- Cavanagh, J.; Fairbrother, WJ.; Palmer, AG.; Rance, M.; Skelton, NJ. *Protein NMR Spectroscopy: principles and practice.* Academic Press; San Diego, CA: 2006.
- Choi HJ, Park-Snyder S, Pascoe LT, Green KJ, Weis WI. Structures of two intermediate filament-binding fragments of desmoplakin reveal a unique repeat motif structure. *Nat Struct Biol.* 2002;9:612–620. [PubMed: 12101406]
- Du Penhoat C, Li Z, Atreya HS, Kim S, Yee A, Xiao R, Murray D, Arrowsmith CH, Szyperski T. NMR solution structure of *Thermotoga maritima* protein TM1509 reveals a Zn-metalloprotease-like tertiary structure. *J Struct Funct Genomics.* 2005; 6:51–62.
- Huang YJ, Hang D, Lu LJ, Tong L, Gerstein MB, Montelione GT. Targeting the human cancer pathway protein interaction network by structural genomics. *Mol Cell Proteomics.* 2008;7:2048–2060. [PubMed: 18487680]

- Jansson M, Li Y-C Jendeborg L, Anderson S, Montelione G, Nilsson B. High-level production of uniformly ^{15}N -and ^{13}C -enriched fusion proteins in *Escherichia coli*. *J Biomol NMR*. 1996;7:131–141. [PubMed: 8616269]
- Liu CG, Maercker C, Castann MJ, Hauptmann R, Wiche G. Human plectin: organization of the gene, sequence analysis, and chromosome localization (8q24). *Proc Natl Acad Sci USA*. 1996;93:4278–4283. [PubMed: 8633055]
- Moseley HNB, Monleon D, Montelione GT. Automatic determination of protein backbone resonance assignments from triple resonance nuclear magnetic resonance data. *Methods in Enzymology*. 2001; 339:91–108. [PubMed: 11462827]
- Raman S, Lange OF, Rossi P, Tyka M, Wang X, Aramini J, Liu G, Ramelot TA, Eletsky A, Szyperski T, Kennedy MA, Prestegard J, Montelione GT, Baker D. NMR structure determination for larger proteins using backbone-only data. *Science*. 2010;327:1014–1018. [PubMed: 20133520]
- Shin SJ, Smith JA, Rezniczek GA, Pan S, Chen R, Brentnall TA, Wiche G, Kelly KA. Unexpected gain of function for the scaffolding protein plectin due to mislocalization in pancreatic cancer. *Proc Natl Acad Sci U S A*. 2013; 110:19414–19419. [PubMed: 24218614]
- Tugarinov V, Kay LE. Ile, Leu, and Val Methyl Assignments of the 723-Residue Malate Synthase G Using a New Labeling Strategy and Novel NMR Methods. *J Am Chem Soc*. 2003;125:13868–13878. [PubMed: 14599227]
- Wishart D, Sykes B. The ^{13}C Chemical-Shift Index: A simple method for the identification of protein secondary structure using ^{13}C chemical-shift data. *J Biomol NMR*. 1994;4:171–180. [PubMed: 8019132]

(a)

(b)

Domain architecture of human protein plectin with domain 6 (PRD6) being highlighted in grey **b** 2D [^{15}N , ^1H] HSQC spectrum recorded at 750 MHz ^1H resonance frequency and 25 °C in ~1 hour for a 0.8 mM solution of PRD6-NCD-ILV. Resonance assignments were obtained for 195 out of 196 expected backbone signals (excluding the N-terminal tag for purification) and are indicated using the one-letter amino acid code. **c** 2D ct [^{13}C , ^1H] HSQC spectrum (ct delay 28 ms; Table I) recorded in ~1 hour for PRD6-NCD-ILV. Resonance assignments of methyl group resonance assignments are indicated, and stereo- specifically assigned methyl groups of Val and Leu are in italics. In cases of signal overlap, stereo-specific assignments were obtained with reference to additional spectra recorded with longer ct delays (Table I; Du Penhoat et al. 2005).

Table I

NMR data acquisition parameters

Sample	Experiment	Dimension $t_{\max}(\text{ms})$; Complex points			Measurement time (hrs)
PRD6- NCD-ILV	2D [^{15}N , ^1H] HSQC	$\omega_2(^1\text{H})$: 57;512	$\omega_1(^{15}\text{N})$: 50;200		1
	2D ct [^{13}C , ^1H] HSQC	$\omega_2(^1\text{H})$: 57;512	$\omega_2(^{13}\text{C})$: 27;407		1
	3D TROSY-HNCA	$\omega_3(^1\text{H})$: 57;512	$\omega_2(^{13}\text{C})$: 9;50	$\omega_1(^{15}\text{N})$: 25;69	18
	3D TROSY-HN(CO)CA	$\omega_3(^1\text{H})$: 57;512	$\omega_2(^{13}\text{C})$: 9;60	$\omega_1(^{15}\text{N})$: 25;69	44
	3D TROSY-HNCO	$\omega_3(^1\text{H})$: 57;512	$\omega_2(^{13}\text{C})$: 16;50	$\omega_1(^{15}\text{N})$: 27;78	6
	3D TROSY-HN(CA)CO	$\omega_3(^1\text{H})$: 57;512	$\omega_2(^{13}\text{C})$: 16;60	$\omega_1(^{15}\text{N})$: 25;69	44
	3D TROSY-HNCACB	$\omega_3(^1\text{H})$: 57;512	$\omega_2(^{13}\text{C})$: 6;92	$\omega_1(^{15}\text{N})$: 24;68	38.5
	3D TROSY-HN(COCA)CB	$\omega_3(^1\text{H})$: 57;512	$\omega_2(^{13}\text{C})$: 6;92	$\omega_1(^{15}\text{N})$: 24;68	48
	3D HMC[CG]CBCA	$\omega_3(^1\text{H})$: 57;512	$\omega_2(^{13}\text{C})$: 9;104	$\omega_1(^{13}\text{C})$: 14;60	19
	3D HMCBCA	$\omega_3(^1\text{H})$: 57;512	$\omega_2(^{13}\text{C})$: 9;104	$\omega_1(^{13}\text{C})$: 14;60	19
	3D [(H)C]-NOESY-[CH], ($\tau_{\text{mix}} = 250$ ms)	$\omega_3(^1\text{H})$: 57;512	$\omega_2(^{13}\text{C})$: 24;100	$\omega_1(^{13}\text{C})$: 12;50	45
	3D [H]-NOESY-[NH/CH] ($\tau_{\text{mix}} = 250$ ms)	$\omega_3(^1\text{H})$: 57;512	$\omega_2(^1\text{H})$: 24;220	$\omega_1(^{13}\text{C})$: 12;52 $\omega_1(^{15}\text{N})$: 18;52	41
PRD6-NC	2D [^{15}N , ^1H] TROSY [#]	$\omega_2(^1\text{H})$: 40;512	$\omega_1(^{15}\text{N})$: 44;128		0.6
	2D ct [^{13}C , ^1H] HSQC, aliphatic [#]	$\omega_2(^1\text{H})$: 40;512	$\omega_1(^{13}\text{C})$: 24;428		0.5
	3D (H)CCH-COSY, aliphatic [#]	$\omega_3(^1\text{H})$: 47;512	$\omega_2(^{13}\text{C})$: 6;108	$\omega_1(^{13}\text{C})$: 6;36	10
	3D (H)CCH-TOCSY, aliphatic [#]	$\omega_3(^1\text{H})$: 47;512	$\omega_2(^{13}\text{C})$: 6;108	$\omega_1(^{13}\text{C})$: 6;36	10
	3D [H]-NOESY-[NH/CH] ($\tau_{\text{mix}} = 70$ ms) [#]	$\omega_3(^1\text{H})$: 47;512	$\omega_2(^1\text{H})$: 17;183	$\omega_1(^{13}\text{C})$: 6;54 $\omega_1(^{15}\text{N})$: 18;54	53
PRD6-NC5	2D ct [^{13}C , ^1H] HSQC (ct delay 28 ms)	$\omega_2(^1\text{H})$: 57;512	$\omega_1(^{13}\text{C})$: 28;184		4
	2D ct [^{13}C , ^1H] HSQC (ct delay 42 ms)	$\omega_2(^1\text{H})$: 57;512	$\omega_1(^{13}\text{C})$: 42;277		5.5
	2D ct [^{13}C , ^1H] HSQC (ct delay 56 ms)	$\omega_2(^1\text{H})$: 57;512	$\omega_1(^{13}\text{C})$: 56;369		7.5

[#] Acquired on the Bruker Avance 900 MHz spectrometer of the Cleveland Center for Membrane and Structural Biology, Case Western Reserve University, OH